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ULTRASTRUCTURAL AND CYTOCHEMICAL CHANGES IN THE RAT LIVER DURING ADAPATION TO HYPOXIA

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Normobaric hypoxia is currently being used on an ever-increasing scale in curative and preventive medicine in connection with various pathological conditions [2]. Hypoxia leads to rapid disappearance of intracellular glycogen in the liver and other organs, due to its mobilization as a source of energy during anaerobic glycolysis. Under these circumstances fatty acids (FA) become more important as energy substrates [4, 5], although for their combustion in the Krebs cycle under these conditions considerable obstacles are created in the form of a deficient oxygen supply to the cells and associated disturbances of the structure and function of the main energy-producing structures, namely the mitochondria. The question arises: what structural and functional mechanisms in animal and human tissues are responsible for maintaining energy homeostasis, and thereby ensuring a favorable preventive and therapeutic effect through the use of interrupted normobaric hypoxia?

The aim of this investigation was to answer the above question by parallel cytochemical and electron-microscopic methods. The organ chosen as the test object was the liver – the main organ in which carbohydrates and FA, the principal energy substrates of the body, are formed and stored during metabolism.

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EXPERIMENTAL METHOD

Experiments were carried out on 15 male Wistar rats weighing 200 ± 20 g, of which five underwent adaptation to hypoxia (experimental) and 10 remained intact (control). Adaptation of the animals to a hypoxic atmosphere was carried out in 0.06-m^3 pressure chamber under normobaric conditions. The hypoxic atmosphere contained 10 ± 1% of oxygen and 90 ± 1% of nitrogen (HO-10). The HO-10 was supplied at the rate of 60 liters/min. The gas mixture in the chamber was monitored by means of an OA-250 gas analyzer (England). A distinguishing feature of the hypoxic training was that the supply of HO-10 was cyclic (5 min inhalation of HO-10, followed by 5 min of inhalation of atmospheric air). This program simulates the natural biorhythm of pO_2 in animals [6]. The course of adaptation to hypoxia consisted of inhalation of the hypoxic mixture for 2 h daily under the above-mentioned conditions for 10 days. The animals were then killed by decapitation and their liver removed for investigation. Tissue samples were taken from the substance of the middle lobe of the liver. They were fixed successively in paraformaldehyde and osmium tetroxide, dehydrated in alcohol and acetone, and embedded in a mixture of equal volumes of Epon and Araldite Semithin and ultrathin sections were cut on the LKB-8800 Ultrotome. Ultrathin sections were stained with lead acetate and uranyl acetate, and examined under the JEM-7A electron microscope. The semithin sections were stained with methylene blue for general survey purposes and by the PAS reaction according to McManus to detect glycogen. With this latter method it is possible to detect not only glycogen, but also fat simultaneously in sections due to the use of osmic acid as the main component of the fixative for the liver tissue. Quantitative assay of fat and glycogen in the hepatocytes was carried out by morphometry of these structures in semithin sections, using Avtandilov's counting grid [1].

EXPERIMENTAL RESULTS

Morphometric analysis of the content of lipid inclusions in the hepatocytes in semithin sections through the liver revealed a marked decrease in their number after the end of the course of adaptation to hypoxia. In the control rats this amounted to 2.87 conventional units (100%) compared with 0.9 (31%) in the experimental rats. The glycogen content in the hepatocytes of the trained rats, on the other hand, rose sharply, on average fourfold, and amounted to 4.2 conventional units (100%) compared with 18 (429%) respectively. An increase was found not only in its proportion by volume in the cytoplasm of the hepatocytes, but also in the intensity of staining during histochemical testing for this polysaccharide.

The pink color of the hepatocyte nuclei in the trained animals is noteworthy (they were pale in the control rats). This may be both an indication of the appearance of intranuclear glycogen and a sign of stimulation of functional activity of the nuclei, for the Schiff's reagent, a component of the stain for glycogen, can also stain DNA.

It must also be pointed out that the sinusoids in the liver of the experimental rats were wider than in the control.

Ultrastructural changes in the liver of all the experimental rats on electron-microscopic investigation were found to be identical.

Interrupted hypoxia appreciably increased the number of microparticles (peroxisomes) and lysosomelike structures in the hepatocytes. Many of these organelles contained electron-dense particles, similar in size and shape to the β-granules of glycogen (Fig. 1a). A second feature of the action of hypoxia on the hepatocytes was the universal hyperplasia and hypertrophy of the smooth endoplasmic reticulum. Some of its dilated cisterns were electron-translucent, others were filled with a homogeneous substrate of average electron density (Fig. 1b). Some cisterns contained electron-dense small granules or chains of them, resembling in structure the γ- and β-glycogen (Fig. 1c). Comparison of ultrathin and semithin sections showed that the most intense PAS-positive reaction for polysaccharides in the hepatocytes corresponded to the structures indicated in Fig 1a-c.

Typical electron-dense lipid inclusions were not present in the cytoplasm of the hepatocytes of the experimental rats. However, cavities surrounded by glycogen were frequently found (Fig. 1d), and were formed at the site of the resorbed fat.

The rough endoplasmic reticulum did not differ in its structure from that in the control rats. It was represented by densely packed membranes with ribosomal granules located on their outer side (Fig. 2). However, they occupied a larger area in the cytoplasm of the hepatocytes of the trained rats than in the control.

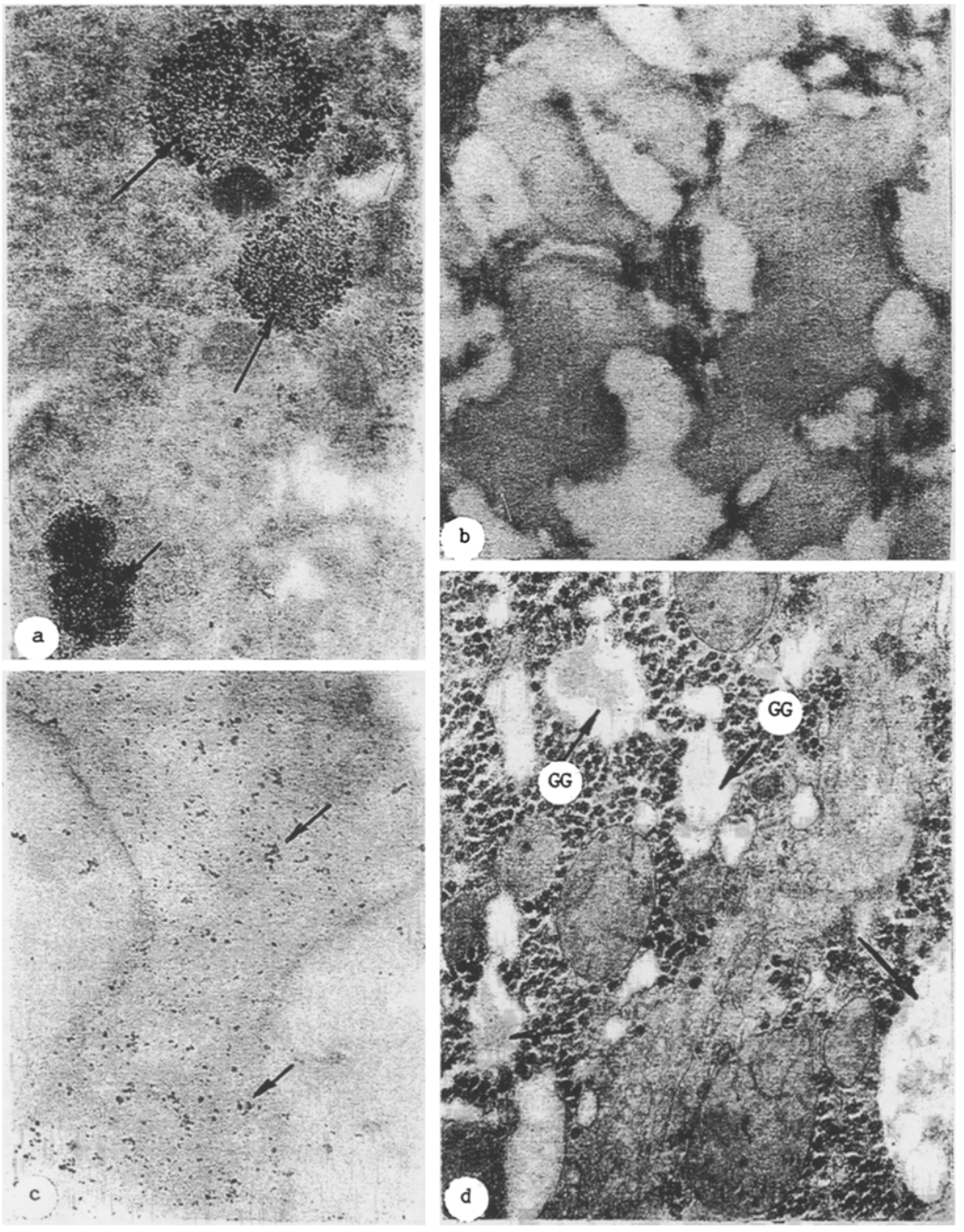

Fig. 1. Subcellular topography of glyconeogenesis in hepatocytes of rats adapted to hypoxia: a) glycogen in microparticles (arrow), magnification 30,000; b) hypertrophied cisterns of smooth endoplasmic reticulum with pale and gray contents, magnification 15,000; c) glycogenlike structures inside dilated cistern of smooth endoplasmic reticulum (arrow), magnification 40,000; d) concentration of glycogen granules (GG) around cavities formed at site of resorbed fat (arrow), magnification 20,000.

The structural organization of the mitochondria of the hepatocytes in rats adapted to interrupted hypoxia was virtually identical with that in the control rats (Fig. 3; see also Fig. 2). In acute hypobaric hypoxia marked swelling of these organelles took place, with clearing of the matrix and destruction and disappearance of the cristae [3]. This was not observed in the rats we studied at the end of the course of interrupted normobaric hypoxia.

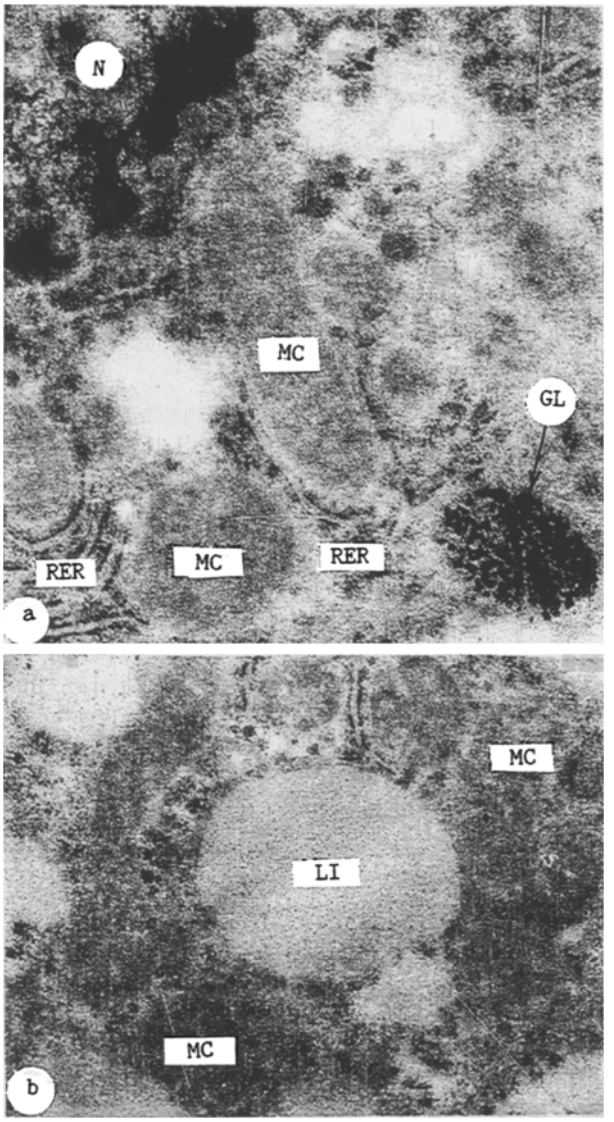

Fig. 2. Fragment of hepatocyte of rat adapted to hypoxia (a) and control intact rat (b). FI) Fat inclusion, GL) glycogenosome, RER) rough endoplasmic reticulum, N) nucleus, MC) mitochondria (magnification 30,000).

Hepatocyte nuclei of the adapted rats were distinguished by their large size and by signs of enhanced functional activity: diffuse chromatin, large nucleoli, adjacent to the karyolemma, in which there were numerous pores. Hepatocytes with two nuclei were often seen. Granules differing from granules of ribonucleoproteins by their increased contrast were found in many nuclei. They were probably glycogen granules, for they were found in nuclei which were pink in semithin sections stained for glycogen.

Another point to note is that interrupted hypoxia appreciably increased adhesion of the blood cells to the wall of the sinusoids.

The above results of electron microscopy and cytochemistry are unambiguous evidence of metabolic correlation between the content of fat and glycogen in the hepatocytes. In the experimental rats disappearance of fat deposits in the cytoplasm of the hepatocytes took place simultaneously with an increase in their glycogen content, and also with hyperplasia of the peroxisomes, in many of which glycogenlike granules were found. More recently, an important role in β-oxidation of FA in mammalian liver cells has been ascribed to peroxisomes [8]. The number of publications reporting functioning of a glyoxylate cycle in the microparticles of liver cells of higher animals, through

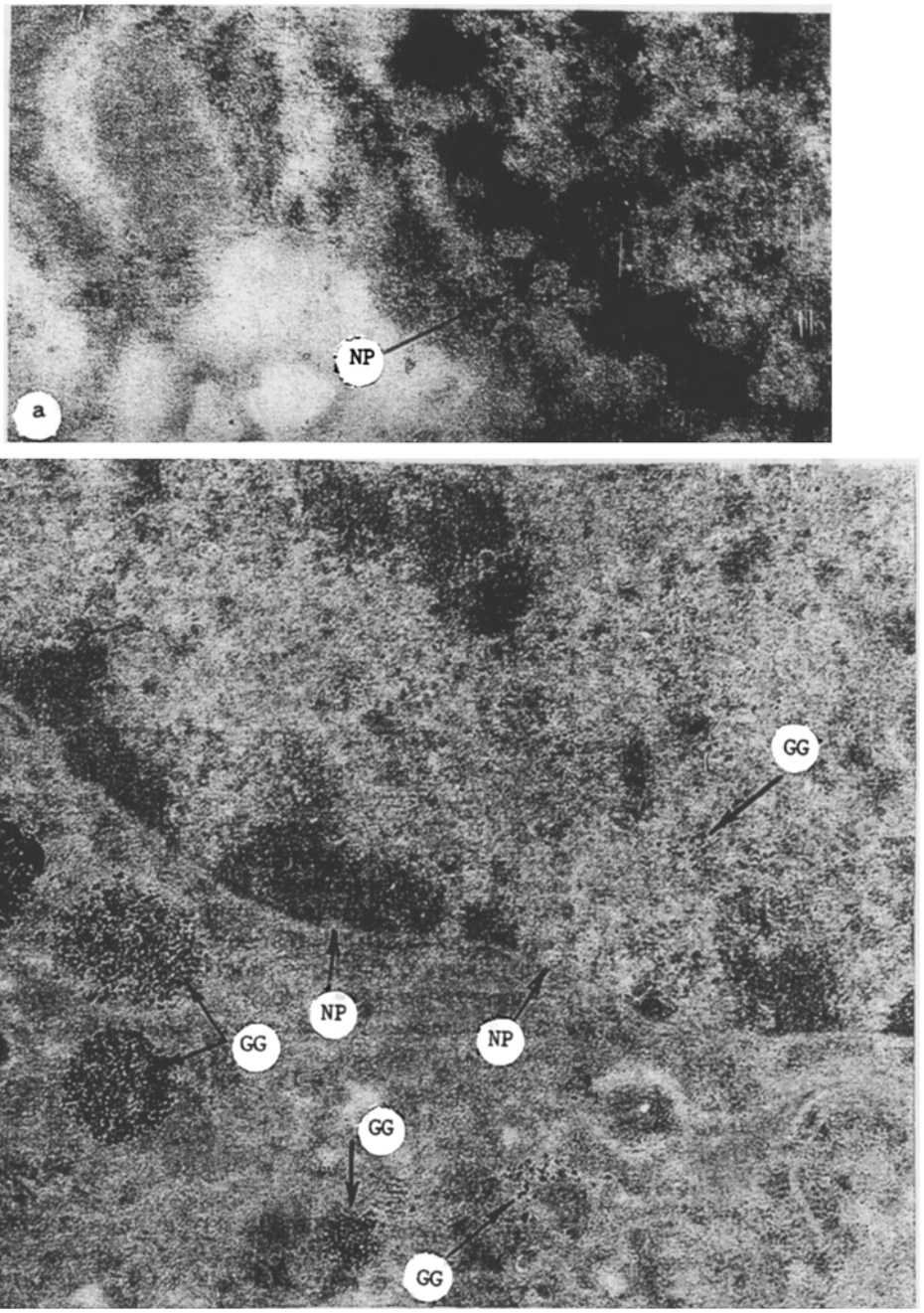

Fig. 3. Functionally active nuclei in rat hepatocytes: a) fragment of nucleus (tangential section), b) glycogenlike granules (GG) in nucleus, the same granules in cytoplasm and microparticles. NP) Nuclear pores. 30,000×.

the intervention of which glucose and glycogen can be synthesized from breakdown products of FI [7], also has increased.

The formation of carbohydrates from FA can also take place by omega-oxidation of the latter [9]. This method of gluco- and glyconeogenesis is connected structurally with the so-called microsomal system of the cells, the main components of which are membranes of the smooth endoplasmic reticulum. It is probably to omega-oxidation of FA that the glycogenlike granules located within cavities of the smooth endoplasmic reticulum of the hepatocytes of hypoxia-tolerant rats owe their origin, for fatty acids are accumulated by and supplied to these cells [8].

It can be postulated on the basis of the findings described above that the positive prophylactic and therapeutic effects of interrupted normobaric hypoxia are attributable primarily to an increase in the energy stability of the liver cells (probably also the cells of other organs), due to stimulation of anaerobic glycolysis. This stimulation arises through the use of FA as energy substrates in glycolysis after their preliminary conversion into carbohydrates (glucose and glycogen). Switching of energy metabolism of the hepatocytes to the anaerobic type in this way in each of the 5-min intervals of inhalation of the hypoxic mixture removes the functional load from the mitochondria and thereby weakens the action of hypoxia on their structure. As a result, the mitochondria remain capable of undertaking oxidative phosphorylation of substrates by atmospheric air. By causing hyperplasia of the microparticles and membranes of the endoplasmic reticulum, interrupted hypoxia also intensifies the antioxidative activity of the cells, for the microparticles are the main producers of catalase. Nuclear function also is enhanced because of an increase in ribose synthesis through activation of the pentose phosphate pathway of glucose oxidation, which is linked with microsomes. Increased protein-synthesizing activity of the nuclei also creates conditions for the repair of structural defects in the mitochondria, which may arise during exposure to a hypoxic gas mixture, and also for the formation of new mitochondria de novo. Consequently, the rhythm of application of normobaric hypoxia used in the present investigation provides a basis for the rhythmic subcellular reorganization which guarantees energy homeostasis in cells of the liver and also probably. of other organs.

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